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(54) Title: A GENETIC DETERMINANT FOR MALIGNANT MELANOMA

(57) Abstract: The invention provides genetic screens for use in determining susceptibility to malignant melanoma based on determining the presence or absence of one or more polymorphic variants of the XRCC3 gene. Reagent kits for use in such screens are also described.

A GENETIC DETERMINANT FOR MALIGNANT MELANOMA

The present invention is concerned with a genetic factor that may determine an individual's susceptibility to malignant melanoma. In particular, the invention concerns a novel association between a single nucleotide polymorphism in the DNA repair gene XRCC3 and the development of malignant melanoma.

Malignant melanoma is a neoplastic lesion arising from epidermal melanocytes and is a highly invasive and aggressive cancer with a high mortality rate. Its incidence has rapidly increased over the past 30 years. The highest risk is associated with individuals with fair skin who are exposed to intense, intermittent periods of sunlight[1]. Extensive epidemiological and experimental data suggests that ultraviolet radiation is an important environmental carcinogen involved in the initiation and progression of skin cancer[2]. UV-radiation at short wavelengths induces damage in the form of cyclobutane pyrimidine dimers and pyrimidine (6-4) photoproducts. At longer wavelengths it causes single-stranded breaks, DNA-protein crosslinking and generates free-radicals which cause oxidative damage[3]. Cells respond to this damage through the activation of various DNA repair pathways.

DNA repair systems are responsible for maintaining the integrity and of the genome in both somatic and germinal cells by minimising replication errors, deleterious rearrangements arising via aberrant recombination and by removing DNA damage[4,5] which might otherwise result in carcinogenesis. Thus, DNA repair has a critical role in protecting against mutations that can lead to cancer. Absence of incorrect repair can lead to the development of cancer through activation of oncogenes, inactivation of tumour-suppressor genes or loss of heterozygosity.

Repair of damaged DNA involves many proteins performing functions directly on damaged DNA, as well as the interaction and interplay with proteins involved in regulation of DNA replication and progression through the cell cycle[6]. Numerous studies have shown that genes directly involved in DNA repair and maintenance of genome integrity, or genes indirectly involved in the repair of DNA damage through regulation of the cell cycle are critical for protecting against mutations which lead to cancer[4,5,15].

A given DNA repair defect may increase the rate of random mutations or may be specific for mutations associated with a particular carcinogen. In the case of malignant melanoma this would be ultra-violet radiation. The combination of UV-induced DNA damage and defective DNA repair mechanism is therefore likely to significantly increase the risk of developing skin cancer.

Inter-individual variation in DNA repair capacity has been shown through the use of lymphocyte assays. Although findings have been difficult to reproduce, individuals with repair capacity of 65-80% of the population mean are more often in the cancer cohorts[7-14]. Reduced DNA repair capacity constitutes a statistically significant risk factor for breast and lung cancer, with odds ratios ranging from 1.6 to 10.0[8-12,14]. Evidence suggests that the difference in DNA repair capacity among individuals is genetically determined. The phenotype of reduced repair capacity for one pathway is independent of the phenotype for another pathway[15], this is consistent with DNA repair being genetically regulated. Measurement of repair capacity in twins[16] and the elevated frequency of individuals with reduced repair capacity among relatives of cancer patients is further evidence that repair capacity is a genetic trait[8,10,11,17]. This variation in DNA repair capacity has characteristics

expected of cancer susceptibility genes; the proteins encoded by these alleles exhibit reduced function rather than absence of function, which causes disease. They exist at polymorphic frequency in the general population and they exhibit incomplete penetrance[5,18]. Thus malignant melanoma in part may be caused by intermittent intense ultra-violet exposure of skin genetically ill-adapted to deal with it.

One mechanism which the inventors suspect may lead to inter-individual variation in DNA repair capacity is genomic variation within the DNA repair genes. A number of polymorphisms in genes that encode DNA repair proteins have been described[18]. These genes, XRCC1, ERCC1, XPD, XPF and XRCC3, encode enzymes involved in three DNA repair pathways, known to be involved in the correction of UV-induced DNA damage. Many of the variants result in amino acid substitutions and exist at polymorphic allele frequencies (i.e. allele frequencies >0.05). Given the known relationship of DNA repair to cancer, polymorphic variants in the DNA repair enzymes have the potential to be population risk factors for cancer because of the large number of individuals affected.

In order to investigate any possible associations between polymorphic variation in genes encoding DNA repair enzymes and development of malignant melanoma the inventors have developed a PCR-SSP strategy to simultaneously genotype a large number of different DNA repair gene polymorphisms under identical conditions and have used this strategy to perform an association study in a cohort of malignant melanoma patients and a control population. The results of this study indicate a significant association between the presence of at least one variant 'T' allele at position 18067 of the XRCC3 gene (exon 7) and development of melanoma ($p=0.004$; OR 2.36).

Therefore, in accordance with a first aspect of

the invention there is provided a method for determining whether an individual is likely to be susceptible to malignant melanoma which comprises screening the genome of said subject for the presence or absence of one or more polymorphic variants of the XRCC3 gene.

The method of the invention preferably comprises screening the genome of the individual for one or more polymorphic variants of the XRCC3 gene which have previously been demonstrated to show statistically significant association with susceptibility to malignant melanoma, for example in a population-based genetic association study. Most preferably, the polymorphic variant will result in an amino acid substitution which may affect the function of the protein product encoded by the XRCC3 gene.

In a preferred embodiment the method of the invention comprises determining the genotype of the individual at position 18067 in exon 7 of the XRCC3 gene, wherein individuals having one or more T alleles at this position are scored as likely to be susceptible to malignant melanoma.

As will be illustrated in the accompanying Example, a single nucleotide polymorphism at position 18067 in exon 7 of the XRCC3 gene (GenBank Accession No. GSDB:S:1297788) has been shown to be associated with the development of malignant melanoma. The common allele is 18067 'C', whilst the variant allele associated with the development of malignant melanoma is 18067 'T'. The variant 'T' allele occurs at a frequency of 0.38[18] and results in the non-conservative amino acid substitution T241M. The invention also contemplates screening for the presence of a protein product of the XRCC3 gene carrying this amino acid substitution.

In accordance with a second aspect of the invention there is provided a method for determining

whether an individual is likely to be susceptible to malignant melanoma which comprises determining the genotype of the said individual at two or more polymorphic loci selected from position 18067 in exon 7 of the XRCC3 gene, position 30028 in exon 11 of the XPF gene and position 2063 in the 5'UTR of the XPF gene, wherein individuals having three or more T alleles at these polymorphic loci are scored as likely to be susceptible to malignant melanoma.

Most preferably, the method of the invention would involve determining the genotype of the individual at all three of the above-listed polymorphic loci.

In addition to demonstrating a significant association between the XRCC3 exon 7 18067 T allele and development of malignant melanoma, the present inventors have also demonstrated a strong additive effect of the XRCC3 exon 7 18067 T allele and the XPF exon 11 or XPF 5'UTR T alleles on the development of melanoma. Although neither of these XPF alleles are observed to show significant association with melanoma when considered alone, it appears that individuals having a total of three or more T alleles at these loci have increased risk of developing melanoma as compared to individuals carrying 0-2 T alleles.

In view of the complexity and number of enzymes involved in DNA repair it is likely that the genetic component of an individual's DNA repair capacity is the aggregate of many 'minor' gene effects. Thus, an individual's total genetic risk for developing UV-induced malignant melanoma may result from the presence of a combination of gene polymorphisms, an example of this being the observed additive effect of the XRCC3 exon 7 18067 T allele and the XPF exon 11 or XPF 5'UTR T alleles.

In view of the foregoing, it is within the scope of the invention to perform screens for the presence

or absence in the genome of the human individual of one or more polymorphic variants of the XRCC3 gene, for example the 18067 T allele, in conjunction with screens (in the same individual) for other polymorphisms associated with malignant melanoma, for example as part of a panel of screens.

As would be readily appreciated by one of ordinary skill in the art, the further polymorphisms associated with malignant melanoma need not necessarily all be single nucleotide polymorphisms but might include other types of polymorphic variation such as, for example, variable number tandem repeats. Moreover, the further polymorphisms need not necessarily occur in genes involved in DNA repair pathways but could be any polymorphic variation associated with melanoma. The further polymorphisms will preferably be ones for which a statistically significant association with malignant melanoma has been demonstrated, for example in a population-based association study. However, it will be appreciated that the panel might also include screens for polymorphic variants which are either in linkage disequilibrium with or in close physical proximity to a marker shown to be associated with melanoma but which have not themselves been shown to be associated with melanoma in a population-based study.

As would be readily apparent to persons skilled in the art of human genetics, "linkage disequilibrium" occurs between a marker polymorphism (e.g. a DNA polymorphism which is 'silent') and a functional polymorphism (i.e. genetic variation which affects phenotype or which contributes to a genetically determined trait) if the marker is situated in close proximity to the functional polymorphism. Due to the close physical proximity, many generations may be required for alleles of the marker polymorphism and the functional polymorphism to be separated by

recombination. As a result they will be present together on the same haplotype at higher frequency than expected, even in very distantly related people. As used herein the term "close physical proximity" means that the two markers/alleles in question are close enough for linkage disequilibrium to be likely to arise.

In accordance with the invention, the process of "determining the genotype" of an individual at a specific position of a given gene may advantageously comprise screening for the presence or absence in the genome of the subject of both the common allele and the variant allele or may comprise screening for the presence or absence of either individual allele, it generally being possible to draw conclusions about the genotype of an individual at a polymorphic locus having two alternative allelic forms just by screening for one or other of the specific alleles.

The step of determining the genotype of an individual at a given polymorphic locus, also referred to herein as 'genotyping', can be carried out using any suitable methodology known in the art and it is to be understood that the invention is in no way limited by the precise technique used to perform such genotyping.

Known techniques for the scoring of single nucleotide polymorphisms (see review by Schafer, A. J. and Hawkins, J. R, ref 35) include mass spectrometry, particularly matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS, ref 36), single nucleotide primer extension[37,38] and DNA chips or microarrays[39,40]. The use of DNA chips or microarrays could enable simultaneous genotyping at many different polymorphic loci in a single individual or the simultaneous genotyping of a single polymorphic locus in multiple individuals.

In addition to the above, SNPs are commonly

scored using PCR-based techniques, such as PCR-SSP using allele-specific primers (described by Bunce, 1995, ref 20). This method generally involves performing DNA amplification reactions using genomic DNA as the template and two different primer pairs, the first primer pair comprising an allele-specific primer which under appropriate conditions is capable of hybridising selectively to the wild type allele and a non allele-specific primer which binds to a complementary sequence elsewhere within the gene in question, the second primer pair comprising an allele-specific primer which under appropriate conditions is capable of hybridising selectively to the variant allele and the same non allele-specific primer.

A still further technique for scoring SNPs is the so-called PCR ELISA technique. SNPs may also be scored by DNA sequencing.

If the SNP results in the abolition or creation of a restriction site then genotyping can be carrying out by performing PCR using non-allele specific primers spanning the polymorphic site and digesting the resultant PCR product using the appropriate restriction enzyme (also known as PCR-RFLP). Restriction fragment length polymorphisms, including those resulting from the presence of a single nucleotide polymorphism, may also be scored by digesting genomic DNA with an appropriate enzyme then performing a Southern blot using a labelled probe corresponding to the polymorphic region (see Molecular Cloning: A Laboratory Manual, Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

The known techniques for scoring polymorphisms are of general applicability and it would therefore be readily apparent to persons skilled in the art that known techniques could be adapted for the scoring of any of the single nucleotide polymorphisms mentioned

herein, such as the XRCC3 18067 C/T polymorphism.

As would be readily apparent to those skilled in the art, genotyping is generally carried out on genomic DNA prepared from a suitable tissue sample obtained from the subject under test. Most commonly, genomic DNA is prepared from a sample of whole blood, according to standard procedures which are well known in the art.

The methods described herein provide simple and straightforward genetic screens which may be used to identify 'at risk' individuals who may be more susceptible to the development of malignant melanoma by virtue of their genetic make-up. The ability to identify 'at risk' individuals using these genetic screens may allow intervention with strategies aimed at reducing the impact of a high risk genotype-environment interaction in these individuals. The 'at risk' individuals might be advised to reduce their exposure to environmental factors known to be associated with melanoma, for example by avoiding prolonged and/or repeated exposure to sunlight, or to take precautionary measures, such as the application of sunscreen. 'At risk' individuals may also be primary candidates for new anti-melanoma therapies, such as topical application of DNA repair enzymes [34] and possibly gene therapy.

In a still further embodiment, the present invention provides a kit for use in determining the genotype of an individual at position 18067 in exon 7 of the XRCC3 gene, particularly in the context of any of the specific genetic screens mentioned herein, the kit comprising at least an oligonucleotide comprising 10 or more contiguous nucleotides from the human XRCC3 gene, including the polymorphic locus at position 18067.

Also provided is a kit for use in performing simultaneous genotyping at two polymorphic loci

selected from position 18067 in exon 7 of the XRCC3 gene, position 30028 in exon 11 of the XPF gene and position 2063 in the 5'UTR of the XPF gene, the kit comprising at least two oligonucleotides selected from:

i) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XRCC3 gene, including the polymorphic locus at position 18067;

ii) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XPF gene, including the polymorphic locus at position 30028; and

iii) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XPF gene, including the polymorphic locus at position 2063 in the 5' UTR.

The oligonucleotide molecules for inclusion into these kits are preferably from 10 to 50 nucleotides in length, even more preferably from 15-30 nucleotides in length. Skilled artisans will appreciate that the precise length of the oligonucleotide and positioning of the polymorphic nucleotide may vary depending upon the nature of the technique to be used to perform genotyping at the polymorphic locus. For example, PCR-SSP generally requires allele-specific primers in which the polymorphic nucleotide is positioned at the extreme 3' end (see list provided in the accompanying Example), whereas techniques based on hybridisation might require allele-specific oligonucleotide probes having the polymorphic nucleotide positioned towards the middle of the probe.

Oligonucleotides for inclusion into the kit may be synthesised using chemical synthesis techniques well known in the art. The oligonucleotides may be DNA, RNA or a synthetic nucleic acid and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will

be readily appreciated by those skilled in the art. Possible modifications include, for example, the addition of isotopic or non-isotopic labels, substitution of one or more of the naturally occurring nucleotide bases with an analog, inter-nucleotide modifications such as uncharged linkages (e.g. methyl phosphonates, phosphoamidates, carbamates, etc.) or charged linkages (e.g. phosphorothioates, phosphorodithioates, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence to form a stable hybrid. Such molecules are known in the art and include, for example, so-called peptide nucleic acids (PNAs) in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The oligonucleotide molecules for inclusion into the kit are preferably single stranded and may correspond to the sense strand or the antisense strand of the relevant gene and to either allelic variant.

In a specific embodiment, the invention provides kits of primers for use in performing methods of the invention using PCR-SSP.

The present invention will be further understood with reference to the following Example, together with the accompanying Figures in which:

Figure 1 is a graphical representation of the relationship between genotype frequencies for the XRCC3 exon 7 18067 C/T polymorphism and the risk of malignant melanoma. The black bars represent the cases from the malignant melanoma patient cohort, the white bars represent the controls.

Figure 2 graphically illustrates the additive effect of the XRCC3 and XPF alleles for the total study group. The vertical bars represent the number of

individuals carrying 0-4 risk alleles. The black bars represent the cases from the malignant melanoma patient cohort, the white bars represent the controls. The P value (p=0.006) gives the statistical significance level of the risk of developing malignant melanoma between those individuals carrying 0-2 alleles and those carrying 3-4 'risk' alleles.

Example

Selection of candidate genes

Ten polymorphisms in the DNA repair genes XRCC1, XPD, ERCC1, XPF and XRCC3 were studied [18]. All polymorphisms within the 5' UTR and those resulting in amino acid changes were studied. Two further polymorphisms were included as they may be in linkage disequilibrium with uncharacterised functional polymorphisms. A list of the genes selected for analysis is provided in Table 1, below:

Table 1: Genes selected for analysis with positions of single-nucleotide substitutions according to GenBank accession number (in brackets) with resulting amino acid change.

Gene (GenBank accession No.)	*Exon*	*Position of polymorphism*	*Nucleotide substitution*	*Amino acid change*
XRCC1	6	26304	C→T	Arg-Trp
(L34079)	10	28152	G→A	Arg-Gln
XPD	6	22541	C→A	No change
(L47234)	10	23591	G→A	Asp-Asn
	23	35931	A→C	Lys-Gln
XPF	5'UTR	2063	T→A	No change
(L76568)	11	30028	T→C	No change
ERCC1 (M63796)	4	19007	G→A	No change
XRCC3	7	18067	C→T	Thr-Met
(GSDB:S:1297788)	5'reg	4541	A→G	No change

Patient cohorts

The patient cohort comprised of 125 individuals with histologically confirmed malignant melanoma. Patients were referred to the regional cancer centre if they had lesions or staging suggesting a high risk of relapse or if they had metastatic disease. All were caucasian.

The control population consisted of 211 caucasoid cadaveric renal transplant donors. The representative nature of this cohort of the UK population has been demonstrated in multiple HLA typing reports [19].

Genotyping assay

The polymerase-chain reaction technique (PCR-SSP) was used to genotype the polymorphisms of interest under universal conditions. This methodology has previously been applied to other polymorphic genes including those of the HLA complex[20]. Genomic DNA extraction[21], PCR amplification and gel electrophoresis[20] was conducted as previously described. DNA amplification was carried out using primers specific for the selected DNA repair polymorphisms, as listed in Table 2:

Table 2: List of primers for PCR-SSP

Gene	Exon	Primer Sequence	SEQ ID NO:
XRCC1	6 pos 26304-C	5' ggggATgTCTTgTTgATCCg	1
	6 pos 26304-T	5' ggggATgTCTTgTTgATCCA	2
	6 pos consensus	5' ggCCTTCTCCCTgcctct	3
	10 pos 28152-G	5' CgTgTTgAggCCTTACCTCC	4
	10 pos 28152-A	5' CgTgTTgAggCCTTACCTCT	5
	10 pos consensus	5' CACCTCATgTAggCTTgCg	6
XPD	10 pos 23591-G	5' CACCCTgCAgCACTTCgTC	7
	10 pos 23591-A	5' CACCCTgCAgCACTTCgTT	8
	10 pos consensus	5' TCCCCggCCCCCCAgATC	9

	6 pos 22541-A	5' gCCTgCCCCACTgCCgA	10
	6 pos 22542-C	5' gCCTgCCCCACTgCCgC	11
	6 pos consensus	5' AAgTATgggCACCAgCCCT	12
	23 pos 35931-A	5' gAATCAgAggAgACGCTgA	13
	23 pos 35931-C	5' gAATCAgAggAgACGCTgC	14
	23 pos consensus	5' CggACCCTCAgCgCCAg	15
ERCC1	4 pos 19007-G	5' gCCAAATTCCCAgggCACg	16
	4 pos 19007-A	5' gCCAAATTCCCAgggCACA	17
	4 pos consensus	5' CAgACCCggggACCCTTT	18
XPF	5' UTR 2063-T	5' gAgTCggCTTCCTTCggCT	19
	5' UTR 2063-A	5' gAgTCggCTTCCTTCggCA	20
	5' UTR consensus	5' CTTCTCggggCCCCTCC	21
	11 pos 30028-T	5' TgACTCgggAAgggTTTCA	22
	11 pos 30028-C	5' TgACTCgggAAgggTTTCg	23
	11 pos consensus	5' gTgCgTggAgCgCAAgAg	24
XRCC3	7 pos 18067-T	5' CAgTCCCTgggggCCAT	25
	7 pos 18067-C	5' CAgTCCCTgggggCCAC	26
	7 pos consensus	5' CCTCCATggCCTCTGTCAC	27
	5' reg 4541-A	5' TgggCCCTCTgTgCACAT	28
	5' reg 4541-G	5' TgggCCCTCTgTgCACAC	29
	5' reg consensus	5' ggTCTTgCAgTgggAgAgA	30

Statistical analysis

Allele, genotype and phenotype frequencies were calculated. Alleles at each polymorphism were said to be in Hardy-Weinberg equilibrium if the observed homozygote and heterozygote frequencies did not differ significantly (p.0.05) from those expected when analysed by Chi-square.

Associations were assessed using contingency table analysis and the Chi-square test (with Yates' correction) using KnowledgeSEEKER (Angoss Software Corporation, Toronto, Canada). Odds ratio and the

relative risk was calculated and a Bonferroni correction of 10 (number of polymorphisms investigated) was used to correct for multiple comparisons.

Results

Phenotype, allele and genotype frequencies were calculated for the 10 DNA repair gene polymorphisms in the melanoma and control cohorts (Table 3).

Analysis of the DNA repair phenotype revealed an association with the T/C polymorphism at position 18067 of exon 7 of the XRCC3 gene and the development of melanoma. Those individuals with the T allele had a significantly increased risk of developing melanoma compared to those without this allele, odds ratio 2.36[1.44-3.86], p=0.0004 (adjusted by Bonferroni's correction factor to p=0.0004) (Table 4 and Figure 1).

In addition, an association was found with the development of melanoma and the TT genotype at exon 11 position 30028 of the XPF gene (p=0.04) and the presence of the TT genotype in the 5'UTR polymorphic position of the XPF gene (p=0.04). The associations were reduced in significance once the heterozygote genotype frequencies were included and did not remain significant after Bonferroni correction (Table 5). There did however seem to be a strong additive effect of the XRCC3 exon 7 T allele and the XPF exon 11 or XPF 5'UTR T alleles on the development of melanoma (Figure 2). No association was found with any of the other DNA repair gene polymorphisms studied.

Table 3: Phenotype, allele and genotype frequencies of DNA repair polymorphisms in controls and malignant melanoma cohorts.
(* results which attained significance)

Gene	Polymorphism	Phenotype and allele frequencies					Genotype frequencies		
		Phenotype			Allele			Genotype	
			Controls	Melanoma	Controls	Melanoma		Controls	Melanoma
XRCC1	Exon6 pos23604	c	0.17	0.14	0.09	0.07	cc	0	0
		t	1.00	1.00	0.91	0.93	tt	0.83	0.86
							ct	0.17	0.14
	Exon 10 pos 28152	g	0.91	0.85	0.64	0.61	gg	0.36	0.38
		a	0.64	0.62	0.36	0.39	aa	0.09	0.15
							ag	0.55	0.47
XPD	Exon 6 pos 22541	a	0.67	0.65	0.40	0.38	aa	0.13	0.12
		c	0.87	0.88	0.60	0.62	cc	0.33	0.35
							ag	0.54	0.53
	Exon 10 pos 23591	g	0.87	0.82	0.64	0.61	gg	0.42	0.39
		a	0.58	0.61	0.36	0.39	aa	0.13	0.18
							ag	0.45	0.43
	Exon 23 pos 35931	a	0.85	0.81	0.60	0.60	aa	0.34	0.38
		c	0.66	0.62	0.40	0.40	cc	0.15	0.19
							ac	0.51	0.43
XPF	5'UTR pos 2063	a	0.51	0.38	0.31	0.23	aa	0.11	0.08
		t	0.89	0.92	0.69	0.77	tt	0.49	0.62*
							at	0.40	0.30
	Exon 11 pos 30028	t	0.88	0.90	0.67	0.74	tt	0.47	0.58*
		c	0.53	0.42	0.33	0.26	cc	0.12	0.10
							tc	0.41	0.32
ERCC1	Exon 4 pos 19007	a	0.82	0.85	0.55	0.60	aa	0.27	0.35
		g	0.73	0.65	0.45	0.40	gg	0.18	0.15
							ag	0.55	0.50
XRCC3	Exon 7 pos 18067	c	0.89	0.83	0.70	0.57	cc	0.52	0.31
		t	0.48	0.69	0.30	0.43	tt	0.11	0.17*
							ct	0.37	0.52
	5'region pos 4541	a	0.42	0.42	0.77	0.78	aa	0.04	0.04
		g	0.96	0.96	0.23	0.22	gg	0.58	0.58
							ag	0.38	0.38

Table 4: analysis of the DNA repair phenotypes showed that the T allele in position 18067 of the XRCC3 gene was significantly associated with malignant melanoma using Chi-squared test.

Gene	Phenotype	Controls	Melanoma	Significance	Corrected
XRCC3 pos18067	Individuals with T allele	102	86	p=0.0004 OR2.36	p=0.004
	Individuals without T allele	109	39	(1.44-3.86)	

Table 5: Analysis of XPF genotype showed that the TT genotype in position 2063 and the TT genotype in position 30028 were significantly associated with development of malignant melanoma. These associations did not remain significant after correcting for multiple comparisons.

Gene	Genotype	Controls	Melanoma	Significance	Corrected
XPF 5'UTR pos2063	Individuals homozygous for TT	104	77	p=0.038 OR 1.65	p=0.38
	Individuals not homozygous for TT	107	48	(1.03-2.66)	
XPF exon 11 pos 30028	Individuals homozygous for TT	98	73	p=0.045 OR 0.62	p=0.45
	Individuals not homozygous for TT	113	52	(1.01-2.60)	

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Claims:

1. A method for determining whether an individual is likely to be susceptible to malignant melanoma which comprises screening the genome of said individual for the presence or absence of one or more polymorphic variants of the XRCC3 gene.

2. A method as claimed in claim 1 which comprises determining the genotype of the said individual at position 18067 in exon 7 of the XRCC3 gene, wherein individuals having one or more T alleles at this position are scored as likely to be susceptible to malignant melanoma.

3. A method for determining whether an individual is likely to be susceptible to malignant melanoma which comprises determining the genotype of the said individual at two or more polymorphic loci selected from position 18067 in exon 7 of the XRCC3 gene, position 30028 in exon 11 of the XPF gene and position 2063 in the 5'UTR of the XPF gene, wherein individuals having a total of three or more T alleles at these polymorphic loci are scored as likely to be susceptible to malignant melanoma.

4. A method for determining whether an individual is likely to be susceptible to malignant melanoma which comprises screening the genome of said individual for the presence or absence of one or more polymorphic variants of the XRCC3 gene and determining the genotype of the said individual at one or more further polymorphic loci associated with development of malignant melanoma.

5. A method as claimed in claim 4 which comprises determining the genotype of the said

individual at position 18067 in exon 7 of the XRCC3 gene.

6. A method for determining the or any genetic basis for malignant melanoma in an individual which method comprises screening the genome of said individual for the presence or absence of one or more polymorphic variants of the XRCC3 gene.

7. A method as claimed in claim 6 which comprises determining the genotype of the said individual at position 18067 in exon 7 of the XRCC3 gene.

8. A method for determining the or any genetic basis for malignant melanoma in an individual which method comprises screening the genome of said individual for the presence or absence of one or more polymorphic variants of the XRCC3 gene and determining the genotype of the said individual at one or more further polymorphic loci associated with development of malignant melanoma.

9. A method as claimed in any one of claims 2, 3, 5 or 7 wherein the step of determining the genotype of an individual at position 18067 in exon 7 of the XRCC3 gene is carried out by PCR-SSP using the primers:

5' CAgTCCCTgggggCCAT
5' CAgTCCCTgggggCCAC
5' CCTCCATggCCTCTGTCAC

10. A method as claimed in claim 3 wherein the step of determining the genotype of an individual at position 30028 of the XPF gene is carried out by PCR-SSP using the primers:

5' TgACTCgggAAgggTTTCA
5' TgACTCgggAAgggTTTCg
5' gTgCgTggAgCgCAAgAg

11. A method as claimed in claim 3 wherein the step of determining the genotype of an individual at position 2063 in the 5' UTR of the XPF gene is carried out by PCR-SSP using the primers:

5' gAgTCggCTTCCTTCggCT
5' gAgTCggCTTCCTTCggCA
5' CTTCTCggggCCCCTCC

12. A kit for use in determining the genotype of an individual at position 18067 in exon 7 of the XRCC3 gene, the kit comprising at least an oligonucleotide comprising 10 or more contiguous nucleotides from the human XRCC3 gene, including the polymorphic locus at position 18067.

13. A reagent kit for use in carrying out the method claimed in claim 3, the kit comprising at least two oligonucleotides selected from:
i) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XRCC3 gene, including the polymorphic locus at position 18067;
ii) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XPF gene, including the polymorphic locus at position 30028; and
iii) an oligonucleotide comprising 10 or more contiguous nucleotides from the human XPF gene, including the polymorphic locus at position 2063 in the 5' UTR.

14. A reagent kit for use in carrying out the method claimed in claim 9, the kit comprising oligonucleotide primers having the following

sequences:

5' CAgTCCCTgggggCCAT

5' CAgTCCCTgggggCCAC

5' CCTCCATggCCTCTGTCAC

15. A reagent kit for use in carrying out the method claimed in claim 10, the kit comprising oligonucleotide primers having the following sequences:

5' TgACTCgggAAgggTTTCA

5' TgACTCgggAAgggTTTCg

5' gTgCgTggAgCgCAAgAg

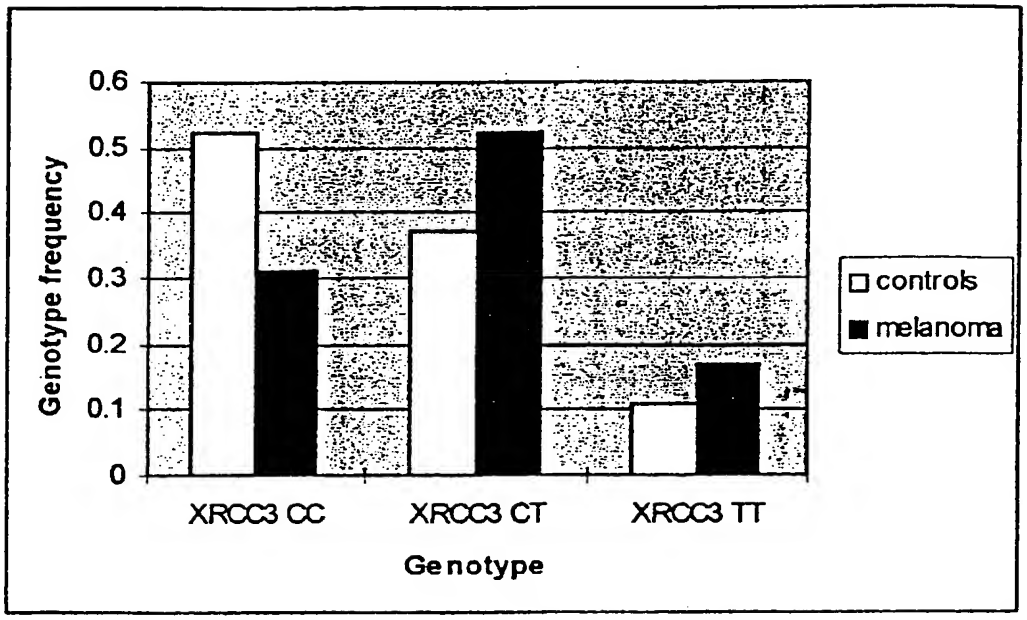
16. A reagent kit for use in carrying out the method claimed in claim 11, the kit comprising oligonucleotide primers having the following sequences:

5' gAgTCggCTTCCTTCggCT

5' gAgTCggCTTCCTTCggCA

5' CTTCTCggggCCCCTCC

FIG. 1.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Genotype frequency
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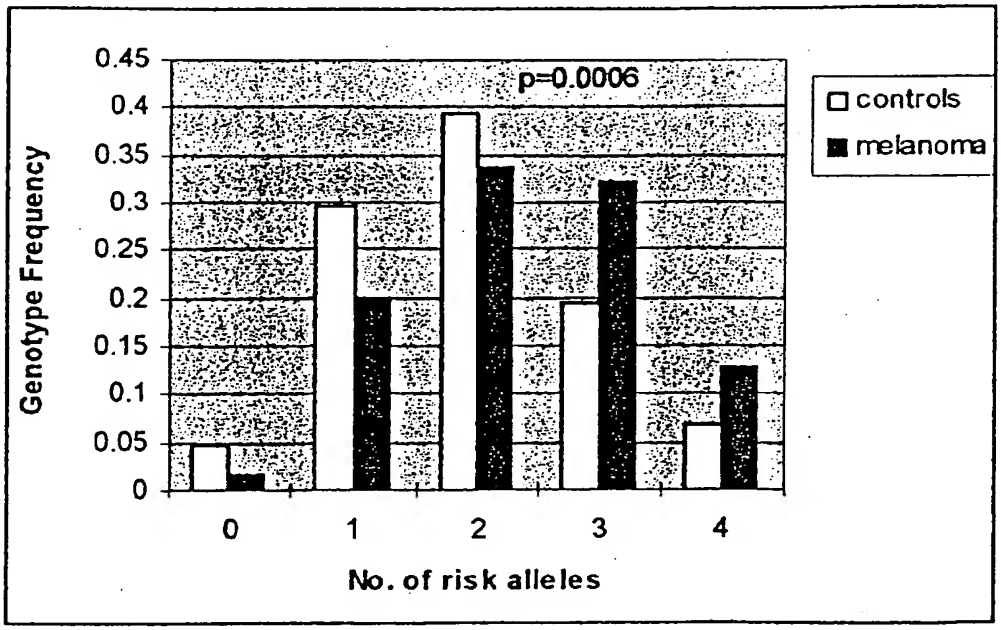
XRCC3 CT
XRCC3 TT
Genotype
controls
melanoma

FIG. 2.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2
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No. of risk alleles
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